ISSN (print) 0093-4666 © 2015. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.165

Volume 130, pp. 165–171 January–March 2015

New taxa of *Ambomucor* (*Mucorales, Mucoromycotina*) from China

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Abstract — A new species and a new variety, *A. ovalisporus* and *A. seriatoinflatus* var. *longior*, are described from soils in northwest China. A key to all known taxa of *Ambomucor* accompanies descriptions and line drawings of the two new taxa. The maximum growth temperature used as an auxiliary criterion in classification of *Mucorales* is also discussed.

Key words — morphology, taxonomy, *Mucoraceae*, *Zygomycota*

Introduction

The genus *Ambomucor* (*Mucorales*), typified by *A. seriatoinflatus* R.Y. Zheng & X.Y. Liu, was introduced recently (Zheng & Liu 2014), together with *A. seriatoinflatus* var. *brevior* R.Y. Zheng & X.Y. Liu and *A. clavatus* R.Y. Zheng & X.Y. Liu. These taxa were found in northwest China. Recently, two new *Ambomucor* taxa were found in the northwest regions (Tibet and Shaanxi Province) of the country and are described here as *A. ovalisporus* and *A. seriatoinflatus* var. *longior*. All *Ambomucor* species and varieties are characterized by simultaneously having two kinds of sporangia: 1) fertile sporangia containing a columella and sporangiospores and 2) aborted sporangia that lack spores and a columella and which proliferate in chains. The criteria for differentiating the taxa of the enlarged *Ambomucor* are almost the same as previously reported (Zheng & Liu 2014). A key to all *Ambomucor* taxa is presented.

Materials & methods

Isolation

A small piece of moss, humus, or dung was soaked and stirred with sterilized distilled water. Different dilutions were poured with PDA media to make plates containing the antibiotic streptomycin. The isolation plates were cultivated in the basement (5–10°C, dark) and at room temperature (about 20°C, natural solar day/night cycle). When colonies were visible under stereomicroscopes, single colonies were transferred to PDA slant tubes for subsequent purification and identification.

Cultures

Vouchers of the two strains studied are preserved in culture collections and the herbarium of the Institute of Microbiology, Chinese Academy of Sciences, Beijing, China: living cultures in the Culture Collection of the State Key Laboratory of Mycology (Am-) and the China General Microbiological Culture Collection Center (CGMCC); and dried cultures in the Herbarium Mycologicum Academiae Sinicae (HMAS).

Media & cultivation

PDA (pH 7) and modified SMA (dextrose 20 g, asparagine 2 g, KH_2PO_4 0.5 g, $MgSO_4{\bullet}7H_2O$ 0.25 g, thiamine chloride 0.5 mg, agar 20 g in 1000 mL distilled water, pH 7; Hesseltine & Ellis 1973) were used for morphological studies. PDA was also used for the mating experiments (with 3% lecithin added) and the detection of maximum growth temperatures. Cultivation durations and temperatures were: 1) 5–7(–10) days at 18°C for describing morphological features; 2) 4–7 days at 25–35°C for determining maximum growth temperatures; and 3) 7–20 days at 18°C for observing sexual reactions. Seven-day cultures in test tubes were used to measure the height of colonies.

Taxonomy

Ambomucor ovalisporus X.Y. Liu & R.Y. Zheng, **sp. nov.** FIG. 1

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Differs from all other *Ambomucor* spp. by its higher colonies, its wider hyphae, its fewer sporangiophore types, its pyriform to obovoid apophysate fertile sporangia, and its globose to oval non-vacuolate sporangiospores.

TYPE: China, Tibet, Nyingchi, Bomê, on wild animal dung, 17 July 2004, Xiao-yong Liu (**Holotype**, HMAS 98956-1; **isotypes**, HMAS 98956-2,-3,-4,-5,-6,-7,-8; ex-type cultures, Am-14, CGMCC 3.06806).

ETYMOLOGY: *ovalisporus*, referring to the oval shape of the sporangiospores.

COLONIES on SMA and PDA at first granulate and then floccose, 9 cm diameter in 7 days at 18°C, up to 2.5 cm high, at first dirty white and then 'Pinkish Buff' (Ridgway XXIX), reverse at first dirty white and then 'Pinkish Cinnamon' (Ridgway XXIX). HYPHAE 21.5–66.5(–80.5) µm diameter, remaining equal or narrowed at septa, hyaline to yellow-brown. STOLONS absent. RHIZOIDS scanty, arising from various parts of the sporangiophores and from fertile and aborted sporangia. SPORANGIOPHORES erect or recumbent, arising directly from substrate or hyphae, forming both fertile and aborted sporangia always in a sympodial pattern, (37–)322–1416(–3476) µm long, 5.5–79 µm diameter, usually equal or subequal in width throughout, always constricted just beneath the terminal fertile sporangia, hyaline to brown, with or without granular contents, always verrucate; main axes mostly simple, single, rarely with 2–3

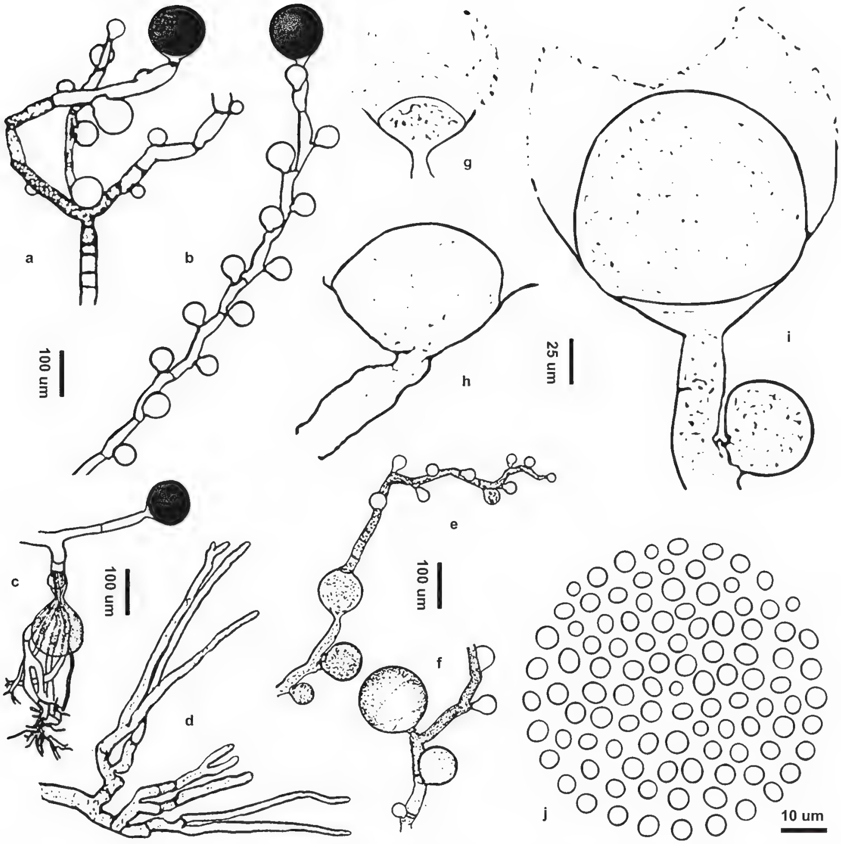

FIGURE 1. *Ambomucor ovalisporus* (CGMCC 3.6806). a, Sporangiophore apex triple branches with an aborted sporangium at the point of separation; b, Single sporangiophore with a fertile top sporangium (in black); c–d, Rhizoids; e–f, Aborted sporangia showing different sizes; g–i, Columellae with distinct collars; j, Sporangiospores. Scale bars: a–f = 100 µm; g–i = 25 um; j = 10 um.

verticillate branches. SEPTA appearing frequently in the hyphae and the main axes of sporangiophores, sporadically in the sporangiophore branches. ABORTED SPORANGIA proliferating repeatedly on the sides of sporangiophores, up to 30 or more, transferring their contents forward and forming a short or long chain of aborted sporangia and terminating in a normal fertile sporangium, pyriform to obovoid and 20–67.5(–83) × 16–58.5(–76) µm, or globose to subglobose and (12.5–)24–85 µm diameter, verrucate, subhyaline. FERTILE SPORANGIA borne terminally on the main axes or the branches of sporangiophores, apophysate, thin-walled, verrucate, globose to subglobose, 26–163.5 µm diameter, dark brown when mature, breaking in larger ones, persistent in smaller ones. COLUMELLAE forming in the fertile sporangia only, well developed, verrucate, with a distinct collar, very regular in shape, hyaline; larger ones subglobose and 102.5–186.5 µm diameter, or oblate and 75.5–92(–179) × 55–73.5(–122) µm; smaller ones applanate and 34.5–39.5 × 26.5 µm. SPORANGIOSPORES forming in fertile sporangia only, released through breaking or moving backward to the sporangiophores, smooth, mostly oval and 6–12.5 × 4.5–9 µm, sometimes globose or subglobose and (5.5–)6.5–9(–10) µm diameter, hyaline. CHLAMYDOSPORES absent. ZYGOSPORES unknown.

COMMENTS: The maximum growth temperature for *Ambomucor ovalisporus* Am-14 was determined as 27°C (results of two tests). Crosses of *Ambomucor ovalisporus* with *A. clavatus* and *A. seriatoinflatus* var. *seriatoinflatus*, var. *brevior*, and var. *longior* all failed to produce zygospores.

Ambomucor seriatoinflatus var. *longior* X.Y. Liu & R.Y. Zheng, var. nov.

MYCOBANK MB518428 FIG. 2

Differs from other *Ambomucor seriatoinflatus* varieties by its higher colonies, its abundant aerial hyphae, its longer narrower sporangiophore branches, and its later sporulation.

TYPE: China, Shaanxi Province, Taibai Mountain, Shangbansi Temple, in moss and humus on rock, 11 October 2002, Xue-wei Wang (**Holotype**, HMAS240174-1; **isotypes**, HMAS 240174-2,-3,-4,-5,-6,-7,-8; ex-type cultures, Am-16, CGMCC 3.014179).

ETYMOLOGY: *longior*, referring to the sporangiophore primary branches being longer than those of *Ambomucor seriatoinflatus* var. *seriatoinflatus*.

COLONIES on SMA and PDA floccose, zonate after 3 days, reaching 9 cm diameter in 6–7 days at 18°C, about 3–5 mm high, at first white, then grayish white, at last grayish white on the edge and 'Dark Purplish Gray' (Ridgway LIII) in the middle, reverse 'Olive-Buff' (Ridgway XL). HYPHAE branched, nonseptate when young, septate in age, 3–7(–8) µm diameter in substrate, abundant in air and (1–)1.5–5(–8) µm diameter. STOLONS absent. RHIZOIDS scanty, not opposite sporangiophores, arising from various parts of the sporangiophores or from the aborted sporangia. SPORANGIOPHORES erect or recumbent, arising directly from substrate or aerial hyphae, forming fertile or aborted sporangia, of three main types: 1) bearing normal fertile sporangia only, 2) aborted sporangia only, or 3) both simultaneously; main axes of the sporangiophores rarely simple, usually branched, 2.9–7.5 µm diameter from substrate, 2–3(–3.5) µm diameter from aerial hyphae; primary branches 1–3, single, in pairs, more often monopodially or pseudoverticillately rebranching, sometimes sympodially and branching no more than three times, (15–)60–543(–1600) µm long, 2–8.5 µm diameter; main axes and branches more or less straight, or subcurved, usually equal or subequal in width throughout, hyaline to pale greenish brown, with

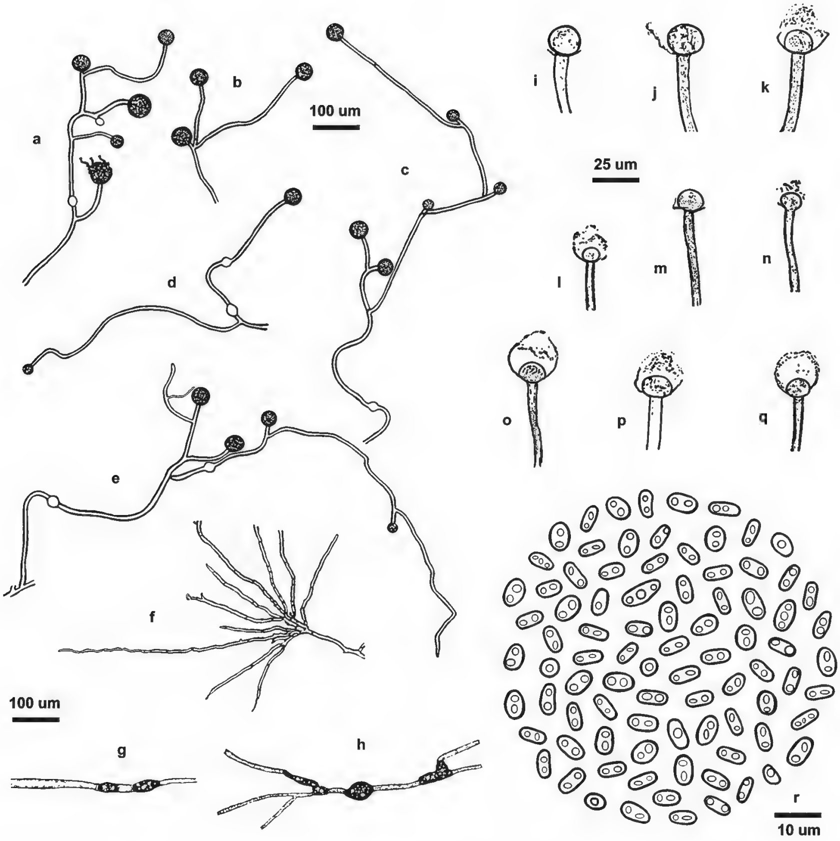

FIGURE 2. *Ambomucor seriatoinflatus* var. *longior* (CGMCC 3.14179). a–e, Upper portions of sporangiophores showing various structures: aborted sporangia (blank) and fertile sporangia (shadowed); f, Rhizoids; g–h, Chlamydospores; i–q, Columellae with distinct collars; r, Sporangiospores. Scale bars: a–h = 100 µm; i–q = 25 µm; r = 10 µm.

or without granular contents, becoming vacuolate in age. SEPTA in main axes and branches of the sporangiophores, usually present at the place of branching, sometimes without definite position. FERTILE SPORANGIA appearing after three days, borne terminally on the main axes or branches of the sporangiophores, globose to slightly depressed globose, (21.5–)24.5–47(–53) µm diameter in larger ones on the sporangiophores from substrate, 7–17 µm diameter in smaller ones on the sporangiophores from aerial hyphae, non-apophysate, breaking or slowly dissolving, thin-walled, dark brown when mature, pale grayish brown in broken pieces of wall. ABORTED SPORANGIA borne terminally on the main axes and branches of sporangiophores, or repeatedly proliferating terminally or laterally, forming intercalary sporangiophores between two aborted sporangia, no more than three times, transferring their contents forward until a fertile and normal sporangium is formed, non-apophysate or slightly apophysate, ovoid and 10–24 × 7–19 µm, globose and 13–22(–26) µm diameter, granulate when young, vacuolate in age, subhyaline. COLUMELLAE formed in fertile sporangia only, well developed, very regular in shape, ovoid to ellipsoid-ovoid, depressed-globose to sub-applanate, 6.5–21 × 5–16.5 µm, hyaline, some with grayish to brownish content, smooth, regularly with a distinct collar. SPORANGIOSPORES forming in fertile sporangia, ellipsoid to broadly oblong-ellipsoid and 3.5–7 × 2–3.5 µm, (1–)2-guttulate, hyaline, grayish in mass, smooth, becoming vacuolate in old cultures. CHLAMYDOSPORES rare, single, or in short chains, subglobose, ovoid or irregular, 5–18.5 × 3–14 µm, hyaline to yellow. ZYGOSPORES unknown.

COMMENTS: The maximum growth temperature for *Ambomucor seriatoinflatus* var. *longior* Am-16 was determined as 30°C (results of two tests). Crosses of *Ambomucor seriatoinflatus* var. *longior* with *A. clavatus*, *A. ovalisporus*, and *A. seriatoinflatus* var. *seriatoinflatus* and var. *brevior* all failed to produce zygospores.

Key to the taxa of *Ambomucor*

(revised from Zheng & Liu 2014)

1. Colonies up to 25 mm high; hyphae 21.5–66.5 µm diameter; both fertile and aborted sporangia borne on the same sporangiophore; fertile sporangia apophysate; sporangiospores globose to oval, avacuolate; maximum growth temperature 27°C .... ***A. ovalisporus***

1. Colonies 1–5 mm high; hyphae 1.5–14 µm diameter; fertile or aborted sporangia or both borne on the same sporangiophore; fertile sporangia non-apophysate; sporangiospores ellipsoid to oblong-ellipsoid, vacuolate; maximum growth temperature no less than 30°C 2

2. Aborted sporangia oval to ellipsoidal; columellae of the fertile sporangia mostly applanate, rarely globose to subglobose; aborted outgrowths frequent, often spirally twisted; maximum growth temperature 33°C .... ***A. clavatus***

2. Aborted sporangia globose, subglobose to ovoid; columellae of the fertile sporangia usually not as above; aborted outgrowths absent or rare, not spirally twisted when present; maximum growth temperature 30°C 3

3. Aborted sporangia borne in chains of up to 20; aborted outgrowths usually absent and not in a zigzag manner; main axes and branches more or less straight, rarely slightly curved .... ***A. seriatoinflatus* var. *seriatoinflatus***

3. Aborted sporangia usually not observed exceeding chains of 6; aborted outgrowths when present formed sympodially; main axes and branches subcurved and rarely straight to sub-straight 4

4. Colonies 3–5 mm high; aerial hyphae abundant; fertile sporangia appearing after three days; aborted sporangia globose when diameter is less than 26 µm; columellae less than 21 µm in width .... ***A. seriatoinflatus* var. *longior***

4. Colonies 1–3 mm high; aerial hyphae scarce; fertile sporangia appearing after one day; aborted sporangia globose, up to 66 µm in diameter; columellae 42 µm or more wide .... ***A. seriatoinflatus* var. *brevior***

Discussion

Based on the results of studies on several genera of *Mucorales*, such as *Actinomucor* (Zheng & Liu 2005), *Cunninghamella* (Zheng & Chen 2001), *Pilaira* (Zheng & Liu 2009), *Rhizomucor* (Zheng et al. 2009), and *Rhizopus* (Zheng et al. 2007), the range of maximum growth temperatures (MGT) is an important criterion for helping circumscribe genera and distinguish species; however, it is not a decisive characteristic and sometimes there is overlap among taxa. The MGTs of the three *Ambomucor* species (*A. ovalisporus* 27°C; *A. seriatoinflatus* 30°C; *A. clavatus* 33°C) occupy a narrow range that supports their generic affinity, but with significant differences that support the species delimitation.

Acknowledgments

The study was partially supported by the National Natural Science Foundation of China (Nos. 31070019 and 31370068), the Ministry of Science and Technology of China (No. 2012FY111600) and the Foundation of the Knowledge Innovation Program of the Chinese Academy of Sciences (No. KSCX2-EW-J-6). We greatly appreciate Associate Prof. Xue-wei Wang of this department for collecting and isolating the type strain of *Ambomucor seriatoinflatus* var. *longior*. We also thank Ms. Hong-mei Liu of this research group for preparing the media and plates, helping with isolation, doing mating experiments, and testing the maximum growth temperatures. Finally we thank Dr. Gerald L. Benny (University of Florida, Gainesville, U.S.A.) and Dr. Bo Huang (Anhui Agricultural University, Hefei, P.R. China) for critical comments and presubmission review.

Literature cited

Hesseltine CW, Ellis JJ. 1973. *Mucorales*. 187–217, in: GC Ainsworth, et al. (eds). The fungi, vol. 4b. Academic Press, New York.

Zheng RY, Chen GQ. 2001. A monograph of *Cunninghamella*. Mycotaxon 80: 1–75.

Zheng RY, Liu XY. 2005. *Actinomucor elegans* var. *meitauzae*, the correct name for *A. taiwanensis* and *Mucor meitauzae* (*Mucorales*, *Zygomycota*). Nova Hedwigia 80: 419–431. http://dx.doi.org/10.1127/0029-5035/2005/0080-0419

Zheng RY, Liu XY. 2009. Taxa of *Pilaira* (*Mucorales*, *Zygomycota*) from China. Nova Hedwigia 88: 255–267. http://dx.doi.org/10.1127/0029-5035/2009/0088-0255

Zheng RY, Liu XY. 2014 ["2013"]. *Ambomucor* gen. & spp. nov. from China. Mycotaxon 126: 97–108. http://dx.doi.org/10.5248/126.97

Zheng RY, Chen GQ, Huang H, Liu XY. 2007. A monograph of *Rhizopus*. Sydowia 59: 273–372.

Zheng RY, Liu XY, Li RY. 2009. More *Rhizomucor* causing human mucormycosis from China: *R. chlamydosporus* sp. nov. Sydowia 61: 135–147.